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Applicant:	Paula M. Vertino
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CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.10

The undersigned hereby certifies that this document is being placed in the United States mail with Express Mail No. EL844533610US, addressed to Commissioner for Patents, Washington, D.C. 20231, on January 18, 2002.

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Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF PAULA M. VERTINO, Ph.D
UNDER 37 C.F.R. § 1.132

I, Paula M. Vertino, state and declare the following:

1. I am the inventor of the above-identified patent application. I make this Declaration in support of the Amendment filed in connection with the above-identified patent application, and in response to the Office Action dated July 17, 2001.

2. This Declaration details the results of experiments carried out under my direct supervision and control. The results relate to an analysis of TMS1 methylation status in normal brain tissue samples, primary glioblastoma multiforme tumor samples, human lung cancer cell lines, and ovarian cancer cell lines. The analysis of TMS1 methylation status in these samples was carried out as described in the specification of the above-identified application.

3. Normal Human Brain Tissue Samples: Normal human brain tissue samples were harvested during autopsy of human subjects not diagnosed with cancer, as conducted at Wesley Woods Geriatric Center, Emory University. These samples were dissected into white and grey matter samples and DNA was isolated. Both white and grey matter samples were tested for TMS1 methylation status. None of the five normal brain tissue samples tested demonstrated TMS1 methylation , in either white or grey matter regions. (See Figure 1, attached.)

4. Primary Human Glioblastoma Multiforme Tumor Samples: Primary human glioblastoma

multiforme tumor samples were collected from patients undergoing surgery at Emory University Hospital between the years of 1998-2000. Seventeen primary glioblastoma multiforme tumor samples were analyzed for TMS1 methylation status. Eight out of seventeen (i.e., 47%) primary glioblastoma multiforme tumor samples analyzed demonstrate TMS1methylation. Figure 2 illustrates eleven samples considered to be representative of the seventeen analyzed. (See Figure 2, samples 2, 3, 6, 8, 9, 10, 11, 12, 13, 14 and 15.) Five of the eleven tumor samples illustrated showed TMS1 methylation. (See Figure 2, samples 2, 8, 9, 14 and 15.) The MCF7 and MB231 cell lines are breast cancer cell lines used as negative and positive controls of TMS1 methylation, respectively. The level of methylation observed in tumor sample 2 is considered to be statistically significant and above the background level of methylation of the MCF7 cell line, which is the negative control.

5. Human Lung Cancer Cell Lines: DNA isolated from human lung cancer cell lines was obtained from Dr. Robert Casero, Johns Hopkins Oncology Center, Baltimore, MD . Fourteen human lung cancer cell lines were analyzed for TMS1 methylation status. (See Figure 3, cell lines H889, H727, H249, H82, N417, A549, H378, U1752, OH3, H69, H157, H125, DMS53 and H460.) Ten of fourteen (i.e., 71%) cell lines demonstrated TMS1 methylation. (See Figure 3, cell lines H889, H727, H249, H82, N417, A549, OH3, H69, H157 and H460.) Cell lines H378, U1752, H125 and DMS53 show no TMS1 methylation. NHBE is a normal bronchial epithelial tissue sample, and it similarly shows no TMS1 methylation.

6. Ovarian Cancer Cell Lines: DNA isolated from thirty one ovarian cancer cell lines was obtained from Dr. Patrice Morin, National Institutes of Aging, Baltimore, MD and analyzed for TMS1 methylation status. (See Figure 4, all samples.) Sixteen of thirty-one cell lines demonstrated significant (i.e., > 10%) TMS1 methylation. (See Figure 4, cell lines A224, A2780, AD10, Bq-1, Es-2, HEY, Hs832, OV1063, OVCA429, OVCAR432, OVCAR-2, OVCAR-3, OVCAR-4, SKOV-3, UC1101 and UC1107.)

7. The data presented herewith demonstrate the presence of TMS1 methylation in tumors and cancer cell lines from brain, lung and ovarian cancer. Accordingly, these data support the assertion in the above-identified application that TMS1 methylation status is a useful marker in a variety of cancers, and is not limited solely to breast cancer.

I , the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

Date: 1/17/02

Paula M. Vertino, Ph.D.